



Superlinkers – A versatile and automatable DNA backbone exchange standard for synthetic biology

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Abstract

Despite the increasing trend to outsource advanced DNA construct synthesis, backbone swapping, e.g. for changing copy number, selection marker or for genomic integration, will likely continue as an important laboratory exercise. Standards in synthetic biology are top priority, but standardizing molecular cloning contrasts flexibility and different researchers prefer and master different molecular technologies. Here, we describe a new, highly versatile and automatable standard “super linkers” for backbone swapping. Super linkers enable backbone swapping with 20 combinations of classical enzymatic restriction/ligation, isothermal (“Gibson”) assembly, ligase cycling reaction, uracil-excision-cloning, Golden Gate cloning and a newly developed ligase-free variant of Golden Gate cloning methodology termed Great Belt cloning (GBC). GBC highly enables automated backbone swapping with simple one-tube protocols directly from plasmid stock solutions. Finally, we demonstrate the value of testing different backbones by demonstrating yield differences from a four-gene pathway with a library of 30 different backbones obtained by combinations of 5 origins and 6 antibiotic resistances derived from pSEVA series.